## **Supporting information**

## **Materials and Methods**

RNA isolation and real-time PCR.

About 1 x 109 bacteria were suspended in RNAprotect bacteria reagent (Qiagen) and pelleted by centrifugation. The cell pellets were stored at -80C. Thawed cells were suspended in 1 ml RLT buffer (Qiagen) containing 2-mercaptoethanol and lysed by bead beating. RNA was purified by using the RNeasy minikit (Qiagen). Contaminating DNA was removed with Turbo DNase (Ambion) and RNA was obtained by using RNeasy MiniElute cleanup kit (Qiagen). cDNA was synthesized with the iScript cDNA synthesis kit (BioRad). Comparative RT-PCR was performed on an ABI Prism 7900 system using SYBR Green PCR MasterMix (Applied Biosystems). Each reaction mixture contained 5 ng of cDNA template in a 25-µL volume. Relative transcript levels were determined by the comparative standard curve method. Standard curves were generated by using gene-specific primers with 10 ng to 0.1 pg of RNA-free genomic DNA purified from B. thailandensis E264. A dissociation curve step was included at the end of each PCR to verify that a single specific product was amplified and to confirm the absence of primer dimers. The glyceraldehyde 3-phosphate dehydrogenase gene BTH 11196 was used as an internal control to verify the absence of significant nonspecific variation between samples.

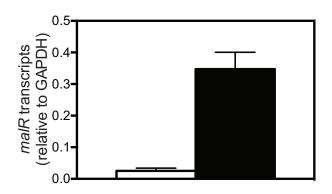


Fig. S1. The effect of endogenous malleilactone production on malR expression.

Real-time PCR was used to measure malR transcript abundance in wild-type B. thailandensis. Logarithmic phase cells were diluted to an  $OD_{600}$  of 0.05 and grown for 8 h without trimethoprim (white bars) or with 15  $\mu$ g/ml trimethoprim (black bars) before collecting cells and isolating RNA. Transcript abundance was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) transcripts. Values represent the mean  $\pm$  range of two independent experiments.

Table S1. Bacterial strains and plasmids

Bacterial strain or plasmid	Strain description	Source or reference
Strains		
B. thailandensis		
E264	Wild type	(1)
BT01447	E264 with an in-frame insertion of the <i>lacZ/hah-Tc</i> transposon in <i>malA</i> after base 5704 out of 8379 with respect to the translation start site	(2)
JBT112	btal1, btal2, btal3 triple mutant of E264	(3)
TT10	JBT112 with the <i>malA-lacZ</i> reporter from BT01447	This study
TT04	BT01447 with a deletion of <i>malR</i>	This study
TT05	BT01447 glmS1 attn7::dhfrllb; TpR	This study
TT06	TT04 glmS1 attn7::plac dhfrllb; TpR	This study
TT11	TT04 glmS1 attn7::plac-malR dhfrllb; TpR	This study
TT07	TT06 with dhfrllb (TpR) marker excised	This study
TT08	TT11 with dhfrllb (TpR) marker excised	This study
E. coli		
DH5α	$F^-$ Φ80 <i>lacZ</i> Δ <i>M15</i> Δ ( <i>lacZYA-argF</i> ) <i>U169</i> hsdR17( $r_K^ m_K^+$ ) recA1 endA1 phoA supE44 thi-1	Invitrogen
MG4	gyrA96 relA1 \(\lambda^-\)	(4)
	$F^ λ^-$ ilvG rfb-50 rph-1 recA Δ(argF-lacIPOZYA)205	
Plasmids		
pJRC115 btaR4	Plasmid used to make <i>malR</i> (formerly <i>btaR4</i> ) deletion in TT10	(3)
pJN105	araC-P <sub>araBAD</sub> cloned into pBBR1MCS-5; Gm <sup>R</sup>	(5)
pQF50	Broad-host-range <i>lacZ</i> fusion vector; Ap <sup>R</sup>	(6)
pFLPe2	Source of Flp recombinase	(7)
pQF50.PmalA	pQF50 containing the <i>malA</i> promoter; Ap <sup>R</sup>	This study
pJN105.malR	pJN105 containing the <i>malR</i> gene; Gm <sup>R</sup>	This study
pQF50.mutPmalA	pQF50.PmalA with the single-base substitutions T3C and G4A in the putative <i>lux</i> box of the <i>malA</i> promoter; Ap <sup>R</sup>	This study
pUC18T-mini-Tn7T-Tp	Mobilizable mini-Tn <b>7</b> vector; Tp <sup>R</sup>	(8)
pUC18-mini-Tn7T-LAC	Mobilizable mini-Tn7 vector with the <i>lac</i> promoter (p <i>lac</i> ) for IPTG-inducible expression of genes; Gm <sup>R</sup>	(8)
pUC18-mini-Tn7T-LAC-Tp	Mobilizable mini-Tn7 vector with p <i>lac</i> ; Tp <sup>R</sup>	This study
pUC18-mini-Tn7T-LAC-Tp-malR	pUC18-mini-Tn7T-LAC-Tp containing the <i>malR</i> gene downstream of plac	This study

## Literature Cited (Table S1).

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